The Clinical Relevance of Pathologic Subtypes in Metastatic Lung Adenocarcinoma

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Introduction: The International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma recommends identification of pathologic patterns in metastatic samples where possible. We investigated the clinical relevance of these patterns.

Methods: Patients with a surgical biopsy of lung adenocarcinoma from a metastatic site were included. Slides were reviewed by an anatomical pathologist identifying the histologic patterns of solid with mucin, acinar, micropapillary, papillary, and assigning a major adenocarcinoma subtype according to the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. *EGFR* and *KRAS* mutation testing were performed on formalin-fixed, paraffin-embedded blocks. Mutations were detected by high resolution melting assay with high resolution melting-positive samples confirmed by Sanger sequencing.

Results: One-hundred patients were included. The major histologic subtype prevalence was as follows: solid (50), acinar (29), micropapillary (20), and papillary (1). Of 100 patients, 45 received no systemic therapy with no overall survival differences seen by histologic subtype and 55 received systemic therapy (chemoradiotherapy with curative intent or palliative chemotherapy). Worse survival was seen in the major solid histologic subtype compared with major acinar (hazard ratio 0.32 [95% confidence interval 0.15–0.68], $p = 0.003$) and micropapillary subtypes (hazard ratio 0.34 [95% confidence interval, 0.17–0.69], $p = 0.003$). The major solid histologic subtype was less likely to harbor *EGFR* mutations ($p = 0.006$) and was less frequent in never smokers ($p = 0.010$) compared with other histologic subtypes.

Conclusion: The major solid histologic subtype of lung adenocarcinoma at metastatic sites is associated with shorter overall survival on systemic anticancer therapy. Furthermore, the major solid histologic subtype is less likely to harbor *EGFR* mutations. These results require validation in larger cohorts.

Key Words: Metastatic lung adenocarcinoma, Histopathology, EGFR, KRAS.

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The morphologic heterogeneity of lung adenocarcinoma has long been recognized. The 2004 World Health Organization (WHO) Classification of Lung Tumors, which is recommended for use in reporting of resection specimens only, identified several different morphologic subtypes of lung adenocarcinoma including acinar, papillary, and solid with mucin patterns and bronchioloalveolar carcinoma.[1] Furthermore, the 2004 WHO classification recommended that where more than one morphologic subtype was present in a tumor, it should be classified as adenocarcinoma of mixed subtype. Over time, it became clear that this diagnostic term, adenocarcinoma of mixed subtype, was of little clinical utility given that nearly 95% of tumors fell under this definition.[2]

In 2011, a new classification for resected lung adenocarcinoma was proposed by the International Association

for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and the European Respiratory Society (ERS).[3] The IASLC/ATS/ERS classification built on previous pathologic studies that demonstrated subtypes with favorable and unfavorable patient outcomes after surgical resection for early stage disease. Multiple independent groups have demonstrated the superiority of the IASLC/ATS/ERS classification over the WHO system in providing prognostic information in patients undergoing resection of early stage lung adenocarcinoma independent of pathologic stage,[4–11] with only one group failing to demonstrate an association.[12]

In addition, for the first time, the new IASLC/ATS/ERS classification provided a classification scheme for reporting of lung cancer in small biopsies and cytology specimens. As part of this proposal, it is suggested that when lung adenocarcinoma is diagnosed in a small biopsy/cytology specimen, the pathologist should "describe (the) identifiable patterns present." Given that there is minimal evidence for this recommendation, the authors of the new classification posed the research question: "In specimens from metastatic sites, is there any clinical significance to recognizing histologic patterns, including the predominant pattern?"[3]

Previously, the distinction between the different subtypes of non–small-cell lung carcinoma in metastatic disease had no bearing on treatment decisions. This has changed recently because of two major discoveries that have had a marked impact on clinical practice. First, patients with metastatic squamous cell carcinoma (SqCC) have been found to be at risk of significant toxicity from bevacizumab caused by major hemorrhage.[13] In addition, patients with metastatic squamous cell carcinoma had inferior survival outcomes, in comparison with those with adenocarcinoma and large cell carcinoma, when treated with pemetrexed-based chemotherapy caused by high expression of thymidylate synthase.[14,15] Second, the prevalence of oncogenic driver mutations, some associated with targeted therapies, differs between adenocarcinoma[16] and squamous cell carcinoma.[17] Review of the literature reveals that only one group has investigated the impact of subtyping of adenocarcinoma in specimens from patients with unresectable lung adenocarcinoma, classifying tumors according to the 1981 WHO classification (the contemporary classification at the time of their study). In this work, the presence of particular adenocarcinoma subtypes had no impact on overall survival (OS).[18,19]

In this study, we aimed to explore the clinical relevance of subtyping adenocarcinoma in biopsy specimens from metastatic sites in patients with metastatic lung adenocarcinoma, according to the new IASLC/ATS/ERS classification. We also examined the relationship of the adenocarcinoma subtypes to mutations in the epidermal growth factor receptor (*EGFR*) and *KRAS* genes.

MATERIALS AND METHODS

Patients

The Human Research and Ethics Committee at St. Vincent's Hospital approved this study. A review of two prospectively maintained clinical databases and associated tumor board meetings was conducted to identify patients who had had surgical sampling of lung adenocarcinoma from a metastatic site between 2000 and 2010. All patients had pathologically confirmed adenocarcinoma defined as a malignant epithelial tumor with histopathologic patterns including acinar, papillary, micropapillary, and solid with mucin adenocarcinoma, defined according to the new IASLC/ATS/ERS classification.[3] Patients included with stage III disease underwent mediastinal sampling for diagnosis and staging, but did not proceed to definitive surgical resection. All identified patients with stage IV lung adenocarcinoma underwent either surgical resection or sampling of a metastatic deposit. Clinical information was collected from the hospital medical records and records of treating medical oncologists and surgeons. The definition of a never smoker was a person with lifetime equivalent consumption of fewer than 100 cigarettes.

Histologic Evaluation

In patients who had undergone multiple surgical procedures for metastatic disease, the case containing the largest amount of tumor tissue was chosen to allow sufficient tissue for *EGFR* and *KRAS* analyses. The size (recorded as the largest dimension of a specimen in millimeters), location, and number of tumors were obtained from the pathology reports. A pathologist reviewed all hematoxylin and eosin (H&E) slides from each case. Patients were excluded at the time of pathologic review if the specimen was very small, if crush artifact was present precluding clear recognition of different adenocarcinoma patterns making it impossible to assign a major histologic pattern, or if there was insufficient available tissue for molecular testing.

The presence of different adenocarcinoma patterns, including acinar, papillary, micropapillary, and solid with mucin, as defined by the new IASLC/ATS/ERS classification, was recorded as a binary variable. It was possible in each case to identify a major histologic pattern; however, it was not possible to assign percentages to the different histologic patterns present. We chose to use the term "major" for the most prominent histologic pattern observed in metastatic samples. This term was used to avoid confusion with the recommendation of the new IASLC/ATS/ERS classification to use the term "predominant" for the most prominent histologic pattern identified in a resection specimen from the lung. All cases underwent immunostaining with TTF1 (clone SPT24, NovoCastra, Newcastle Upon Tyne, United Kingdom).

Molecular Pathology

Deparaffinization and DNA extraction

A formalin-fixed, paraffin-embedded block with adequate tumor for molecular analysis was chosen from each case, and a tumor-rich area was circled on the corresponding glass slide. This region was sampled from each block using two mm diameter dermatology core punches. The punched core tumor tissues were deparaffinized with 800 μl of xylene by incubating for seven minutes, followed by washings with 800 μl of 100 and 70% ethanol. After removal of 70% ethanol, tumor tissues were incubated at 55°C for 15 minutes for removal of residual ethanol. Genomic DNA was extracted using the

DNeasy Tissue and Blood kit (Qiagen, Hilden, Germany) following the manufactures instructions with following modifications based on those of Wu et al.[20] After addition of ATL buffer, tissues were heat treated at 98°C for 15 minutes, followed by incubation at 56°C for 3 days after addition of 36 µl proteinase K (Worthington, NJ) at 20 mg/ml concentration.

EGFR and KRAS mutation testing

EGFR mutations in exons 18 to 21 and *KRAS* mutations in codon 12 and 13 were scanned by high resolution melting using the assay conditions previously described[21,22] with the following modifications. EGFR ex19 F 5′-TAACGTCTTCCTTCTCTCTCTGTC-3′ and EGFR ex19 R 5′-CCCACACAGCAAAGCAGAAACTC-3′ primers were used to amplify a shorter amplicon of 152 bp. To reduce sequence artifacts' arising from uracil because of cytosine deamination in formalin-fixed, paraffin-embedded DNA,[23] 0.5 U of uracil-DNA glycosylase (New England Biolabs) and 0.5X of uracil-DNA glycosylase buffer was added to high resolution melting reaction that was prepared in a final volume of 20 µl. For uracil-DNA glycosylase treatment, an incubation step at 37°C for 30 minutes was added before the standard polymerase chain reaction amplification. Samples were tested in duplicate, and all high resolution melting positives were sequenced using the conditions previously described.[21]

Statistical Analysis

Statistical analysis was undertaken using STATA version 12 (Statacorp LP, TX). Survival analysis was performed using the Kaplan-Meier method with hazard ratios derived using the Cox Proportional Hazards model. Tests of association were performed using Pearson's χ^2 or Fisher's exact test as appropriate. Median times and hazard ratios are reported with their 95% confidence interval (CI). p less than 0.05 was considered statistically significant.

RESULTS

Patient and Tumor Characteristics

This data set of 100 patients, accrued from the years 2000 to 2010, comprised 66% males, with a median age at diagnosis of 64 years (range, 36–86 years). At the time of analysis, 98 patients had died. The Eastern Cooperative Oncology Group (ECOG) performance status at diagnosis was 0 or 1 in 69 patients and ≥ 2 in 31 patients. There were 21 never, 35 current, and 44 former smokers.

In order of frequency, the site of tissue sampling was: brain (30%), pleura (25%), bone/skeletal muscle (20%), mediastinum (18%), and chest wall or supraclavicular fossa (7%). Fifty-two specimens were acquired at metastatectomy, 43 specimens were acquired through open biopsy either as a diagnostic procedure or as part of a therapeutic procedure, and five specimens were core biopsies. The breakdown of specimen type by site together with the median size and size range of specimens is included in Table 1. Treatment was given to patients according to clinician assessment and in keeping with local management guidelines.

Fifteen of 100 (15%) patients presented with unresectable stage III disease at diagnosis and had tissue acquired from a diagnostic mediastinoscopy. Of these 15 patients, 10 received radiotherapy together with platinum doublet chemotherapy with curative intent (66%). One patient (6%) received palliative chemotherapy with carboplatin/gemcitabine. Three patients (20%) received palliative radiotherapy without systemic therapy, and one patient (7%) received best supportive care only. Of the 15 patients, 14 (93%) died as a result of their lung cancer. One patient remains alive at last follow-up of 82 months.

Eighty-five patients presented with stage IV disease at diagnosis. Of these, 44 patients (52%) received systemic therapy. Thirty-eight patients received first-line platinum doublet chemotherapy and three patients received single agent gemcitabine. Two patients had resection of an isolated central nervous system metastasis followed by radiotherapy to the chest and combined concurrent platinum doublet chemotherapy. One patient with an *EGFR* mutation in exon 18 (G719S) and concomitant de novo exon 20 mutation (T790M) received first line afatinib. One patient remains alive at last follow-up of 50 months.

For the 55 patients who received systemic anticancer therapy, the median number of lines of treatment was two (range one to five). Thirty-three patients received two or more lines of therapy. Patients with poor performance status were significantly less likely to receive systemic therapy (ECOG 0–1: 43 of 69 [62%] versus ECOG ≥2: 12 of 31 [39%], χ^2 [1] = 4.82, p = 0.028).

Examples of the major histologic patterns are shown in Figure 1. The most frequent major histologic pattern seen in the 100 metastatic lung adenocarcinoma tumors was solid with mucin at 50%, followed by acinar at 29% and micropapillary at 20%. Major papillary pattern was seen in one case only. The 100 tumors showed a range of different histologic patterns, with the number of different patterns observed in each specimen as follows: one pattern seen in 10% of cases; two patterns in 45%; three patterns in 33%; and four patterns in 12% of cases. The frequency of the different patterns present in individual samples was as follows: solid seen in 82% of cases; micropapillary in 68%; acinar in 68%; and papillary in 29% of cases. Major solid pattern was less common in patients who were never smokers when compared with former or current smokers (Table 1, Fisher's exact test = 0.010). Positive TTF1 immunostaining was present in 84 tumors. No statistical relationship was observed between TTF1 immunostaining and the major histologic pattern.

Sixteen patients had two or more surgically acquired specimens of metastatic lesions that were available for review. Details of the size, site, and patterns seen in these specimens are presented in Table 2. The major histologic pattern was preserved across the different specimens from each patient, but the subtype proportions, particularly for the lesser patterns, varied. For example, one patient (Table 2, patient 2) with resection of one cutaneous and two brain metastases showed a major pattern of acinar with mucin adenocarcinoma but the lesser patterns of micropapillary and papillary adenocarcinoma varied in proportion.

Because of the selection criteria used, only two patients had previous resection of their primary lung tumor. In both

TABLE 1. Sample Site and Method of Acquisition, with Largest Dimension in Millimeters; Associations Between *EGFR* and *KRAS* Mutations with Major Pathologic Subtype; Major Pathologic Subtype and Smoking Status; and *EGFR* and *KRAS* Mutations with Smoking Status

			Metastectomy	Open Biopsy	Core Biopsy		
		N	**(*n*, Mean, Range)**	**(*n*, Mean, Range)**	**(*n*, Mean, Range)**		
Sample site	Brain	30	30, 20 mm, 7–50 mm				
	Pleura	25	6, 42 mm, 26–58 mm	19, 19 mm, 2–75 mm			
	Bone/muscle	20	14, 40 mm, 8–80 mm	2, 34 mm, 28–40 mm	4, 16 mm, 11–22 mm		
	Mediastinum	18	1, 10 mm, N/A	17, 12 mm, 5–30 mm			
	Chest Wall	7	1, 40 mm, N/A	5, 13 mm, 10–16 mm	1, 17 mm, N/A		
		n	Solid	Acinar	MPA	Papillary	
EGFR mutation	Positive	13	2 (15%)	4 (31%)	5 (38%)	1 (8%)	
	Negative	87	48 (55%)	25 (29%)	15 (17%)	0 (0%)	Fisher's exact = 0.006[a]
KRAS mutation	Positive	32	18 (56%)	9 (28%)	5 (16%)	0 (0%)	
	Negative	68	32 (47%)	20 (29%)	15 (22%)	1 (1%)	Fisher's exact = 0.789 [a]
		n	Never Smoker	Former Smoker	Current Smoker		
Major subtype	Solid	50	5 (10%)	24 (48%)	21 (42%)		
	Acinar	29	9 (31%)	5 (17%)	15 (52%)		
	MPA	20	6 (30%)	5 (25%)	9 (45%)		
	Papillary	1	1 (100%)	0 (0%)	0 (0%)	Fisher's exact = 0.010[a]	
EGFR mutation	Positive	13	9 (69%)	4 (31%)	0 (0%)		
	Negative	87	12 (14%)	31 (36%)	44 (51%)	Fisher's exact < 0.001	
KRAS mutation	Positive	32	1 (3%)	17 (53%)	14 (44%)		
	Negative	68	20 (29%)	27 (40%)	21 (31%)	Fisher's exact = 0.006	

[a]Comparison across four histologic groups.
MPA, micropapillary.

patients, the major pattern observed at the metastatic site was concordant with predominant pattern seen in the primary tumor (one patient with major solid pattern and one patient with major acinar pattern).

Correlation of OS With Major Histologic Pattern

Patients receiving no systemic therapy.

There was no difference in OS when correlated with the major histologic pattern in the metastatic site. OS for patients with different major histologic patterns was as follows: major solid pattern was 4.2 months (95% CI 3.3–7.4 months); major acinar pattern was 4.6 months (95% CI 1.5–16.9 months); and major micropapillary pattern was 4.7 months (95% CI 1.5–11.4 months; Table 3, Fig. 2*A*).

The only significant factor influencing OS was ECOG status: median OS ECOG 0 to1 6.3 months (95% CI 4.0–14.1 months), ECOG ≥2: 3.9 months (95% CI 1.8–6.8 months), hazard ratio (HR) 2.1 (1.1–4.0), $p = 0.019$; Fig. 3, Table 3). Analysis stratified according to stage was not possible because of the small number of patients with stage III disease ($n = 4$).

Patients receiving systemic therapy.

Statistically significant differences in OS in patients who received systemic therapy were present by the major histologic pattern. Patients with major solid pattern tumor had worse OS at a median 9.4 months (95% CI 8.6–12.2 months) when compared with patients with major acinar pattern tumor at 15.9 months (95% CI 10.7–24.7 months; HR versus solid 0.32 [0.15–0.68], $p = 0.003$) and patients with major micropapillary pattern tumor at 18.9 months (95% CI 11.6–24.4 months; HR versus solid 0.34 [0.17–0.69], $p = 0.003$; Fig. 2*B*). No difference in OS was seen between treated patients with major acinar and major micropapillary pattern tumors.

No significant differences in OS were identified by the presence or absence of each histologic pattern (Fig. 4). There was a direction of effect towards longer OS in patients with an absence of the solid pattern (solid present [$n = 46$] versus absent [$n = 9$], median OS 11.6 months [95% CI 9.4–14.3 months] versus 17.6 months [95% CI 3.4–44.0 months], HR 2.0 [95% CI 0.98–4.3], $p = 0.056$).

Correlation of Mutational Profile and Major Histologic Patterns

Mutational analysis for *EGFR* and *KRAS* mutations was successful in all 100 patients. *EGFR* and *KRAS* mutations were identified in 13 of 100 tumors (13%) and 32 of 100 tumors (32%), respectively. *EGFR* and *KRAS* mutations were mutually exclusive. *EGFR* mutations occurred most often in major micropapillary pattern tumors (6 of 20 [30%]) followed by major acinar pattern tumors (4 of 29 [14%]) and were least frequent in major solid pattern tumors (2 of 50 [4%]; Fisher's

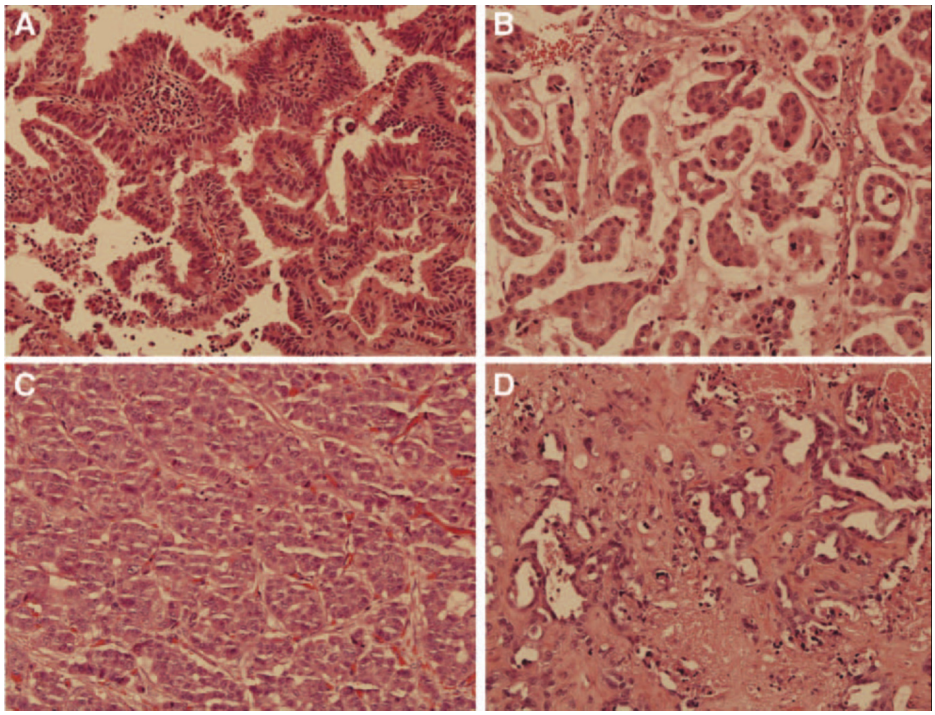

FIGURE 1. Representative photomicrographs of the four histologic patterns seen in the metastatic tumor deposits (H&E, ×200). H&E, hematoxylin and eosin. *A*, Papillary pattern in a station 7 lymph node; (*B*) micropapillary pattern in a brain metastasis; (*C*) solid with mucin pattern in a parietal pleural metastasis; (D) acinar pattern in a brain metastasis

TABLE 2. Histologic subtypes in patients with two or more available samples

	Chosen Lesion			Alternative Lesion(s)		
Patient	**Site, Dimension**	**Major Type**	**Secondary Types**	**Site, Dimension**	**Major Type**	**Secondary Types**
1	Femur, 35 mm	Acinar	Solid, MP	Humerus, 20 mm	Acinar	
2	Brain, 24 mm	Acinar	Pap, MP	Brain, 40 mm	Acinar	Pap, MP
				Skin, surgical scar, 15 mm	Acinar	MP, Pap
3	Femur, 50 mm	Acinar	MP, solid	Femur, 50 mm	Acinar	
4	Brain, 19 mm	MP	Nil	Brain, 15 mm	MP	
5	Femur, 40 mm	MP	Acinar	Femur, 30 mm	MP	
6	L5 mass, 40 mm	MP	Pap, solid	L5 mass, 45 mm	MP	
7	Brain, 25 mm	MP	Pap, solid, acinar	Brain, 27 mm	MP	Solid
				Brain, 12 mm	MP	Solid
				Brain, 25 mm	MP	Solid
8	Pleura, 50 mm	MP	Pap, acinar	Pleura, 25 mm	MP	Acinar, Pap
9	Brain, 25 mm	MP	Solid, Pap	Brain, 7 mm	MP	Solid, Pap
10	Mediastinal LN, 15 mm	Pap	Acinar	Femur, 2 mm	[a]	
11	Brain, 20 mm	Solid	MP	Brain, 8 mm	Solid	MP
				Mediastinal LN, 4 mm	Solid	MP
				Brain, 10 mm	Solid	MP
12	Mediastinal LN, 15 mm	Solid	Acinar, MP	Brain, 19 mm	Solid	Acinar, MP
13	Brain, 25 mm	Solid	MP	Brain, 12 mm	Solid	MP
14	Brain 12 mm	Solid	MP, Pap	Brain, 9 mm	Solid	MP, Pap
15	Brain 10 mm	Solid	Acinar	Brain, 4 mm	Solid	
16	Cervical LN, 16 mm	Solid	MP, acinar	Brain, 7 mm	Solid	Acinar

The largest dimension of each specimen is recorded in millimeters. Secondary types listed by relative proportion.
[a]Scattered atypical cells only.
Pap, papillary; MP, micropapillary; LN, lymph node.

TABLE 3. OS Outcomes

	N	Survival Months (95% CI)	HR (95% CI)	*p*
Systemic therapy	55	13.0 (10.7–16.4)		
Major pattern				
Solid	28	9.4 (8.6–12.2)		
Acinar	13	15.9 (10.7–24.7)	Vs. solid 0.32 (0.15–0.68)	Vs. solid 0.003
Micropapillary	13	18.9 (11.6 - 24.4)	Vs. solid 0.34 (0.17–0.69)	Vs. solid 0.003
Papillary	1	N/A	N/A	
ECOG 0-1	43	13.6 (11.1–17.5)		
ECOG 2 or more	12	8.6 (2.8–17.6)	1.66 (0.9–3.2)	0.128
Male	36	13.0 (9.8–17.2)		
Female	19	11.9 (8.9–20.1)	1.02 (0.6–1.8)	0.944
Stage III	11	13.0 (8.6–24.4)		
Stage IV	44	12.5 (9.5–16.4)	1.56 (0.8–3.1)	0.207
EGFR+	6	17.5 (10.7 to not reached)		
EGFR−	49	12.5 (9.5–15.8)	0.91 (0.4–2.1)	0.827
KRAS+	17	11.6 (6.5–17.2)		
KRAS−	38	13.0 (10.7–17.5)	1.46 (0.8–2.6)	0.209
No systemic therapy	45	4.7 (3.4–7.4)		
Major pattern				
Solid	22	4.3 (3.3–7.4)		
Acinar	16	4.6 (1.9–16.9)	Vs. solid 1.2 (0.5–2.9)	Vs. solid 0.675
Micropapillary	7	4.7 (1.5–11.5)	Vs. solid 0.9 (0.5–1.7)	Vs. solid 0.737
ECOG 0-1	26	6.3 (4.0–14.1)		
ECOG 2 or more	19	3.9 (1.8–6.8)	2.1 (1.1–4.0)	0.019
Male	30	4.2 (3.3–6.3)		
Female	15	11.5 (1.9–16.9)	0.5 (0.3–1.03)	0.062

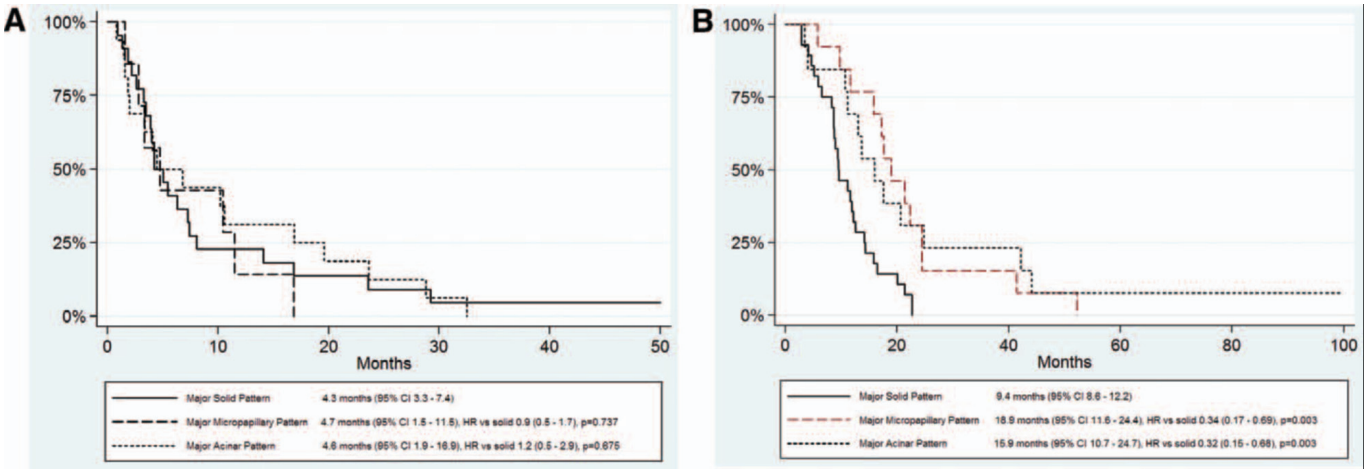

FIGURE 2. Overall survival (OS) by major histologic subtype. *A*, Patients not receiving systemic therapy; (*B*) patients receiving systemic therapy.

exact = 0.006; Table 1). Both patients with major solid pattern tumors and *EGFR* mutations had uncommon variants (patient 1, exon 18 E709_T710delinsD; patient 9, exon 20 insertion), which are associated with resistance to first-generation *EGFR* inhibitors[24] (Table 4). The one major papillary pattern tumor harbored a classic exon 21 L858R mutation. All *EGFR* mutations occurred in never or former smokers and were not present in any current smokers (Fisher's exact $p < 0.001$; Table 1).

KRAS mutations occurred in major solid pattern tumors (18 of 50 [36%]), major acinar pattern (9 of 29 [31%]) and major micropapillary pattern (5 of 20 [25%]) tumors (Table 1), without a significant relationship found between the presence of *KRAS* mutations and the major histologic pattern (Fisher's exact $p = 0.789$, Table 1). Thirty-two *KRAS* mutations were seen with the following frequencies: G12C 20 (63%), G12V 4 (13%), G12D 3 (9%), G12A 2 (6%), G12L 1 (3%), G13C 1 (3%), and

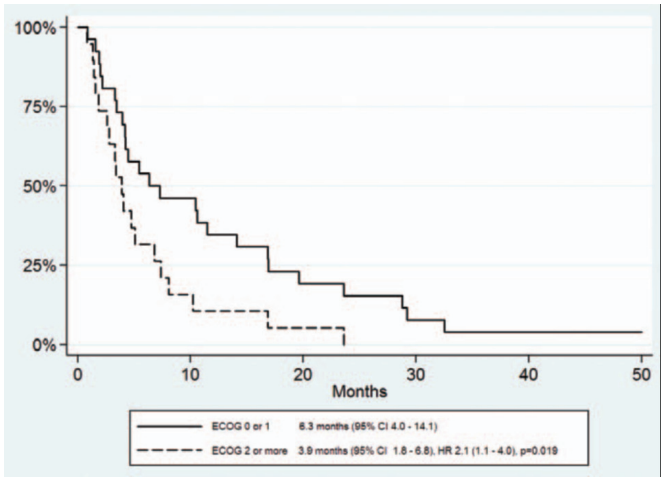

FIGURE 3. Overall survival (OS) by performance status (ECOG) for patients not receiving systemic therapy.

G13D 1 (3%). All patients with *KRAS*-mutant tumors, except one, were former or current smokers (Fisher's exact = 0.006; Table 1). There was no relationship between oncogenic mutations, the major histologic patterns in tumors and OS, which may reflect the small number of patients in our cohort.

DISCUSSION

This study is the first to examine the impact of the histologic pattern present at metastatic sites in lung adenocarcinoma according to recommendation in the IASLC/ATS/ERS classification for small biopsies/cytology specimens.[3] The recommendation states that pathologists should list the histologic patterns present in a small biopsy/cytology specimen, which may be from the primary site or metastatic site. In this study, we followed this recommendation and in addition identified the major histologic pattern in each tumor. A relationship was determined between the major histologic pattern at the metastatic site and OS in patients with unresected stages III and IV lung adenocarcinoma who received systemic chemotherapy. Patients with major solid pattern tumor, who had received systemic therapy, had significantly shorter OS when compared with patients with major acinar and major micropapillary pattern tumors. No significant differences were seen based on major histologic pattern of tumor in patients who did not receive systemic therapy. In addition, we found that *EGFR* mutations are less frequent in major solid pattern tumor

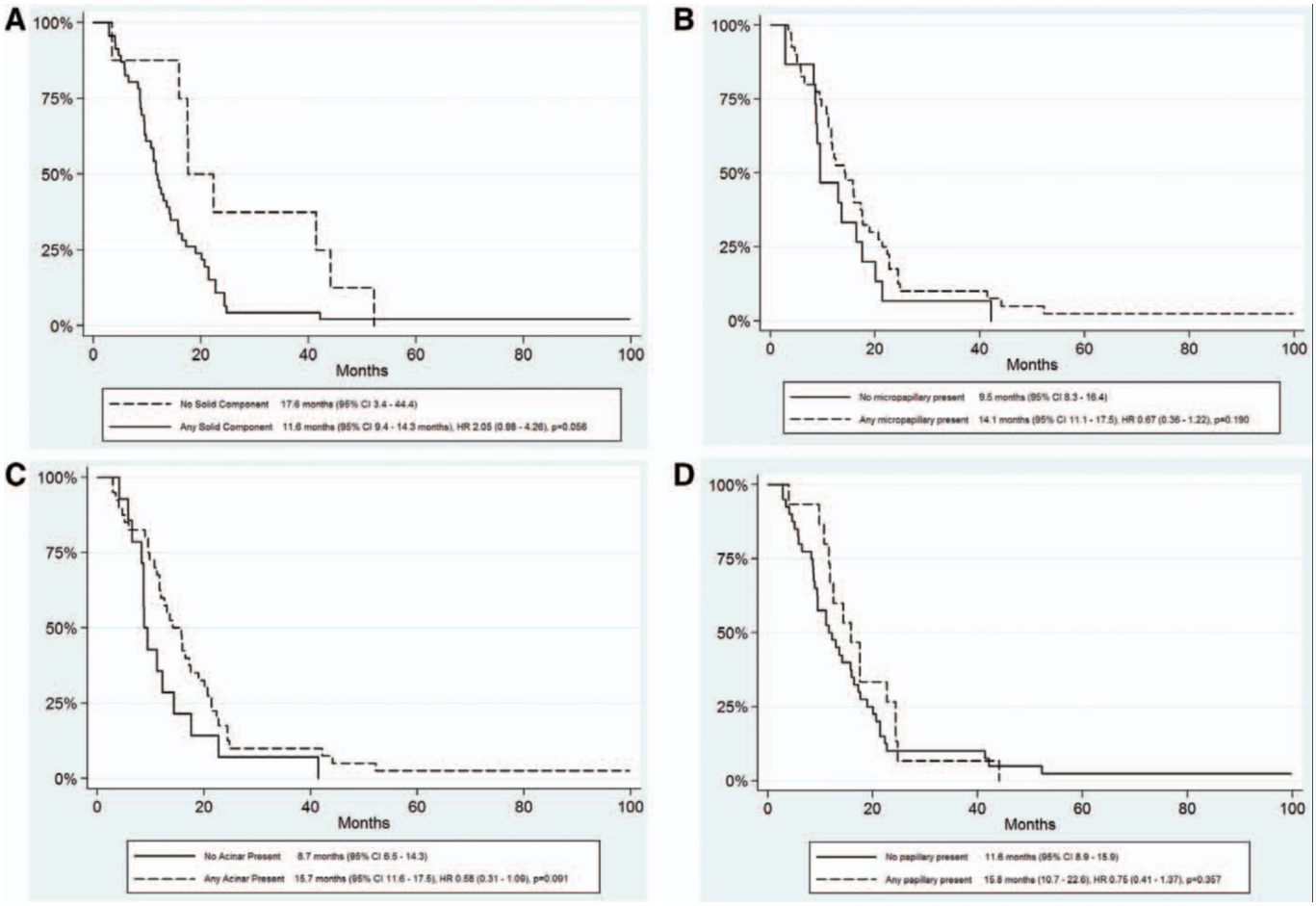

FIGURE 4. Overall survival (OS) by the presence or absence of each pathologic subtype within a specimen. *A*, Solid pattern; (*B*) micropapillary pattern; (*C*) acinar pattern; (*D*) papillary pattern.

TABLE 4. Patients With *EGFR* Mutations

Patient	Age at Diagnosis	Sex	Tumor Site	Smoking History	Major Pattern	Exon	Mutation	Amino Acid Change	EGFR Inhibitor Use and Response
1	83	F	Brain	Former	Solid	18	E709_T710delinsD	c.2127_2129del	Nil
2	45	F	Pleura	Never	Acinar	18/20	G719S / T790M	c.2155G>A and c.2369C>T	Afatinib; six cycles; partial response; CNS progression
3	51	F	Pleura	Never	MPA	19	E746_750del	c.2235_2249del15	Nil
4	68	F	Pleura	Former	MPA	19	E746_L747delinsNY	c.2236_2241delinsAATTAT	Nil
5	54	F	Pleura	Never	Acinar	19	E746_750del	c.2236_2250del15	Nil
6	36	M	Bone/muscle	Never	MPA	19	L747_T751delinsP	c.2239_2251del13insC	Erlotinib; eight cycles; partial response
7	73	F	Bone/muscle	Never	Acinar	19	L747_P753delinsS	c.2240_2258del18	Nil
8	44	M	Pleura	Never	MPA	20	S768_D770dup	c.2303_2311dup	Erlotinib; one cycle; unknown
9	66	M	Pleura	Former	Solid	20	H773_V774insTQPP	c.2318_2319insCACACAACCCCC	Nil
10	55	M	Brain	Never	MPA	21	L858R	c.2573T>G	Nil
11	64	M	Bone/muscle	Never	MPA	21	L858R	c.2573T>G	Gefitinb; 14 cycles; partial response
12	79	M	Pleura	Former	Acinar	21	L858R	c.2573T>G	Nil
13	55	M	Mediastinum	Never	Papillary	21	L858R	c.2573T>G	Erlotinib; six cycles; partial response; CNS progression

M, male; F, female; CNS, central nervous system; MPA, micropapillary.

at metastatic sites in comparison with major micropapillary and acinar pattern tumors.

The role of histologic subtypes/patterns in metastatic lung adenocarcinoma has previously been examined by another group of investigators. In 1989, Sørensen et al. classified the histologic patterns of adenocarcinoma in small biopsies and cytology specimens from either the primary or metastatic site in a group of 220 patients enrolled in a clinical trial according to the 1981 WHO classification (20). This study demonstrated that the median survival of patients with solid pattern tumors was also poor, of the order of 22 weeks; however, this failed to reach statistical significance in comparison with patients with acinar, papillary, and bronchioloalveolar carcinomas, the latter term having been made obsolete in the new IASLC/ATS/ERS classification.[3] Of note, they commented that the bronchioloalveolar carcinoma pattern was not seen in any metastatic tumor. There was a suggestion from their data that partial and complete response rates to chemotherapy were similar across the different histologic subtypes; however, there was a shorter duration of response in patients with solid pattern tumors. It is possible that the lack of a statistically significant difference in OS seen in the cohort from Sørensen et al.[18,19] is a reflection of the activity of the cytotoxic drugs used at the time (vindesine, lomustine, cyclophosphamide, and methotrexate). Patients in this earlier study did not receive the modern platinum based regimens received by many of the patients in our cohort. Given the variability in chemotherapy regimens and differences in the timing of tumor response assessment of patients in our cohort, we were not able to look for differences in response rate.

In a more recent publication from 2012, Warth et al.[5] found differences in response to chemotherapy based on histologic subtype in patients with resected stages III and IV lung adenocarcinoma. They examined the effect of adjuvant chemotherapy for any resected stage and/or adjuvant radiotherapy for resected stages III and IV disease retrospectively in a group of patients. They noted a direction of effect toward patients with resected solid predominant tumors receiving greater benefit from postoperative chemotherapy and/or radiotherapy compared with patients with resected nonsolid predominant tumors. This contrasts with our finding from the purely palliative (unresected) setting that patients with major solid pattern tumors had significantly shorter OS after systemic chemotherapy in comparison with patients with major acinar and major micropapillary pattern tumors. The underlying reasons for this difference are not apparent, but may relate to superior efficacy of chemotherapy agents or radiotherapy in the microscopic or minimal residual disease setting.

Morphologic heterogeneity is well recognized as one of the histologic hallmarks of resected lung adenocarcinoma.[2,3,5] We confirm previous reports that there is also histologic heterogeneity in small biopsy specimens of lung adenocarcinoma from metastatic sites in patients with stages III and IV disease.[25,26] We demonstrate that solid pattern tumor was the most frequent major pattern seen in our cohort of 100 patients, followed by major acinar and major micropapillary patterns, with only one patient with major papillary pattern tumor. The relative frequencies of the major patterns in our cohort differ from relative frequencies of predominant patterns in curative resection specimens reported in recent studies[4–7,11] focusing on the prognostic importance of adenocarcinoma subtypes in resected specimens according to the new IASLC/ATS/ERS classification. In these latter studies, the frequency of the predominant subtype in the primary tumor ranges from 13 to 37.6% for solid

predominant tumors; 13.8 to 45.1% for acinar predominant tumors; 2.3 to 10.3% for micropapillary predominant tumors; and 4.7 to 40.7% for papillary predominant tumors. We surmise that the most likely explanation for this difference is that the frequencies we observed are a function of the metastatic potential of each adenocarcinoma subtype. This has recently been examined by several authors who compared the predominant subtype in the primary tumor with histologic patterns in concurrent metastases and showed that both micropapillary and solid patterns are more likely to be present in metastatic tumors, despite being present as a small percentage in the primary tumor.[25,26] Our cohort only had two patients with resection specimens from their primary lung adenocarcinoma. As such, conclusions cannot be drawn on the relationship between the primary lung adenocarcinoma and its distant metastatic deposits.

The frequency of *EGFR* mutation (13%) in our cohort of 100 patients with unresectable stages III and IV lung adenocarcinoma is low, but consistent with our mostly Western population. *EGFR* mutations were seen mostly in major micropapillary and acinar pattern tumors and in the one major papillary pattern tumor and were infrequent in major solid pattern tumors.

In early stage[27] and stage III[25] cohorts from North America and Australia, respectively, *EGFR* mutations were more frequent in acinar, micropapillary and papillary predominant subtypes and rare in solid predominant subtypes, similar to our cohort of patients with unresectable stages III and IV disease. This observation is unlikely to hold in Asian populations. In some Asian cohorts, a substantial proportion of patients with solid predominant early stage tumors harbor *EGFR* mutations driven by the high prevalence of *EGFR* mutation in lung adenocarcinoma patients from these ethnic groups.[28,29]

KRAS mutations were fairly evenly distributed across all major tumor patterns in our cohort, which is in line with results of Motoi et al.[2] who found no association with predominant pattern and *KRAS* mutation in early stage patients, but contrasts with other resected early stage cohorts where an association between *KRAS* mutations and the solid predominant subtype has been demonstrated.[7,25,27] The known association of *KRAS* mutations and *EGFR* mutations with smoking history was reinforced in this study.

As this was the first exploration of the recommendations from the IASLC/ATS/ERS classification for subtyping metastatic lung adenocarcinoma, our findings require validation in larger cohorts. The number of patients with tissue samples of equivalent size to core biopsies in our study was small. A concern may arise that morphologic subtypes observed on core biopsies are not representative of the major subtype of the tumor. Future studies would need to include such tumor samples to allow greater generalizability of results. Although survival analysis demonstrated worse outcomes for patients with the major solid pattern, the number of patients in both the major micropapillary and major acinar groups was small (n = 13 each). Pooling of these two subtypes confirms worse outcomes for major solid patients (data not shown). The availability of a larger sample size would allow exploration of whether there is a survival difference between major micropapillary and major acinar subtypes.

Given the retrospective nature of this cohort, systemic therapies and response assessment were not standardized. The potential confounding effects of different therapies in our study could be addressed by reviewing the effect of adenocarcinoma subtypes in the setting of a clinical trial where systemic therapies have been standardized.

It is acknowledged that providing increasing detail in histopathologic reports will be time-consuming for clinical pathologists. If others corroborate our findings, the extra detail may provide useful information for clinicians as to the expected clinical behavior while on chemotherapy. In other tumors streams, morphologic findings impact on clinical decision making (e.g., grading in breast[30] and prostate carcinoma)[31] or provide information on the expected clinical behavior and response duration on systemic therapy (e.g., pathologic subtyping in malignant pleural mesothelioma).[32,33] Recognition of different biological behavior among lung adenocarcinoma subtypes may act as stimulus for further basic research to understand the differences and may lead to the discovery of novel targets for new therapeutic approaches.

In conclusion, we have used the morphologic subtypes described in the IASLC/ATS/ERS classification of adenocarcinoma and investigated their clinical relevance at metastatic sites. We have demonstrated that the major solid pattern of adenocarcinoma at metastatic sites is associated with worse OS compared with the major acinar and major micropapillary patterns for patients treated with systemic chemotherapy. We have also demonstrated that major solid pattern is associated with infrequent *EGFR* mutations compared with the major acinar and major micropapillary patterns in this western cohort. No significant association was seen by pathologic patterns with the distribution of *KRAS* mutations.

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